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(54) Bifunctional antibody constructs and method for selectively destroying cell populations.

(57) A bifunctional heterodimeric antibody conjugate comprising (a) an anti-target cell antibody component joined to (b) an anti-lymphocyte antibody component wherein the anti-lymphocyte antibody component is specific f r activated lymphocytes. A bifunctional antibody/cell surface-binding molecule conjugate comprising (a) a molecular component other than an antibody, capable of binding to target cell surface molecules and (b) an anti-lymphocyte antibody component wherein the anti-lymphocyte antibody component is specific for activated lymphocytes. A bifunctional hybrid antibody comprising (a) an anti-target cell binding site component and (b) an anti-lymphocyte binding site component, wherein the anti-lymphocyte binding site component is specific for activated lymphocytes. A method for selectively destroying target cell populations comprising: (1) binding the component (a) of one or more of the above described bifunctional antibody constructs to target cells; (2) activating lymphocytes; and (3) binding the activated lymphocytes to the component (b) of the bifunctional antibody construct; wherein said steps (1), (2) and (3) can be conducted simultaneously or in any order, provided that step (2) is always conducted simultaneously with or prior to step (3).

BIFUNCTIONAL ANTIBODY CONSTRUCTS AND METHOD FOR SELECTIVELY DESTROYING CELL POPULATIONS

FIELD OF THE INVENTION

5   The present invention relates to novel antibody constructs and to a method for selectively destroying cell populations. In particular, the present invention relates to novel bifunctional antibody constructs selected from heterodimeric antibody conjugates (sometimes referred to herein as "antibody heterodimers"), antibody/cell surface-binding molecule conjugates and hybrid antibodies, all of which have dual binding specificity to both activated lymphocytes and cell surface molecules, and to a method for destroying
10   specific target cells, including tumor cells or other diseased cells by recruiting activated lymphocytes such as activated T cells and natural killer cells to attack the target cells selectively by means of the novel antibody constructs.

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BACKGROUND OF THE INVENTION

The use of activated populations of lymphoid cells to lyse or kill tumor targets in the therapy of neoplasia has recently been a subject of intense research interest (Staerz W.D. and Bevan M.J., Immunol.
20   Today 7:241-245 (1986)). Specifically, populations of peripheral lymphocytes, activated by the T-cell derived glycoprotein, Interleukin-2 (IL-2), that is, lymphokine activated killer cells (LAK cells), have been used to successfully treat tumors in vivo in animal models (Mazumler A. and Rosenberg S.A., J. Exp. Med. 159:495-507 (1984)), and recently have been used for the treatment of human tumors resistant to standard therapeutic regimens (Rosenberg S.A. et al., NEJM 316:889-897 (1987)). This form of treatment has been
25   limited, however, by the toxicity of IL-2, which must be given in extremely large doses to be effective (Rosenberg S.A., et al., NEJM 316:889-897 (1987)). Thus, while the concept of using activated lymphoid cells to treat malignancy remains of great interest, attention has turned to potentially less toxic means of in vitro and in vivo activation of these cells.

Lymphoid cells express a variety of lineage-specific surface antigens, some of which are directly
30   involved in the activation of these cells to a cytolytic function (Reiherz E.L., Meuer S.C. and Schlossman S.F., Immunol. Rev. 74:83-112 (1983)). So far, two major surface structures on lymphoid cells have been shown to be involved in this cytolytic activation, namely, the T-cell antigen-receptor/T3 complex (TCR-T3) and the T11 molecule, also known as the E-rosette receptor (Meuer S.C., et al., Cell 36:897-906 (1984) and Oettgen H.C. and Terhorst C., Human Immunol. 18:187-204 (1987)). TCR-T3 is expressed on all mature
35   cytolytic T cells (CTL), and on a few "natural killer" cells (NK cells), and T11 is expressed on both CTL and NK cells (Reinherz E.L., et al., Immunol. Rev. 74:83-112 (1986); Schmidt R., et al., J. Exp. Med. 164:351-356 (1986)). More specifically, certain antibodies to the TCR-T3 complex, or to T11, have been shown to induce and/or enhance killing by CTL or NK cells (Spits H., et al., Eur. J. Immunol. 15:85-91 (1985); Siliciano R.F. et al., Nature 317:428-429 (1985)). Furthermore, it has recently been found that human CTL
40   can be induced to kill irrelevant human tumor targets (i.e., any human target, not just those cells to which the CTL are normally specific) by incubating these CTL and targets in vitro in the presence of bifunctional hybrid antibodies or heterodimeric antibody conjugates which recognize T3 or the T cell receptor (TCR) and a surface determinant on the tumor (Perez P., et al., Nature 316:354-356 (1985); Staerz et al., Nature 314:628-631 (1985) and PNAS 83: 1453-57 (1986); Liu et al PNAS 82:8648-8652 (1985)). Presumably, the
45   hybrid antibody or heterodimeric antibody conjugate allows close cell-cell contact between the T cell and the tumor target by a bridging effect of the bifunctional hybrid antibody or heterodimeric antibody conjugate, with one end of the hybrid antibody or one antibody of the heterodimeric antibody conjugate binding to the tumor cells, and the other end of the hybrid antibody or the other antibody of the heterodimeric antibody conjugate binding to the TCR-T3 complex and activating the CTL. While this
50   approach shows great promise in vitro, the in vivo infusion of such hybrid antibodies or heterodimeric antibody conjugates might be complicated by the fact that the enormous amount of non-activated, normal T cells in the body express T3. These cells would compete with activated T cells for binding to the hybrid antibody or het rodim ric antibody conjugate. Furthermore, the anti-T3/tumor antibody hybrid and conjugates would not activate the majority of NK cells, which are T3 negative.

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SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is to provide a method for selectively destroying cell populations using antibody constructs wherein non-activated normal T cells in the body will not compete with activated T cells for binding to the antibody constructs.

Another object of the present invention is to provide a method for selectively destroying cell populations using antibody constructs wherein a majority of both the CTL cells and NK cells are activated.

An even further object of the present invention is to provide a method of activating and directing lymphocytes to destroy specific cell populations for treatment of certain diseases without harmful side effects.

These and other objects have been attained by providing, in one embodiment of the present invention, a bifunctional heterodimeric antibody conjugate comprising (a) an anti-target cell antibody component joined to (b) an anti-lymphocyte, antibody component wherein the anti-lymphocyte antibody component is specific for activated lymphocytes.

In another embodiment of the present invention there is provided a bifunctional antibody/cell surface-binding molecule conjugate comprising (a) a molecular component, other than an antibody, capable of binding to target cell surface molecules and (b) an anti-lymphocyte antibody component, wherein the anti-lymphocyte antibody component is specific for activated lymphocytes.

In an even further embodiment of the present invention there is provided a bifunctional hybrid antibody comprising (a) an anti-target cell binding site component and (b) an anti-lymphocyte binding site component wherein the anti-lymphocyte binding site component is specific for activated lymphocytes.

The present application also provides a method for selectively destroying target cell populations comprising:

(1) binding the component (a) of one or more of the above described bifunctional antibody constructs to target cells;

(2) activating lymphocytes; and

(3) binding the activated lymphocytes to the component (b) of the bifunctional antibody construct; wherein said steps (1), (2) and (3) can be conducted simultaneously or in any order, provided that step (2) is always conducted simultaneously with or prior to step (3).

BRIEF DESCRIPTION OF THE FIGURE

The Figure gives Fluoresence-Activated Cell Sorter (FACS) patterns showing the binding of J5, anti-T11$_2$, anti-T11$_3$, and conjugates J5-anti-T11$_2$ and J5-anti-T11$_3$ to CALLA-expressing and T11$_2$/T11$_3$-expressing cells: Namalwa cells (groups a through h) or REX cells (groups i through p) incubated with no antibody (a,i), J5 (b,j), anti-T11$_2$ (c,k), anti-T11$_3$ (d,l), anti-T3 (e,m), J5-anti-T11$_2$ (f,n), J5-anti-T11$_3$ (g,o) or J5-anti-T3 (h,p).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention provides an "alternative activation" pathway using the T11 molecule, presenting several advantages over the above described prior TCR-T3 pathway of activation. First, the T11 molecules are present on all NK cells as well as CTL cells, and the appropriate anti-T11 antibodies or T11 binding site as part of (a) a heterodimeric antibody conjugate, (b) antibody/cell surface-binding molecule conjugate or (c) bifunctional hybrid antibody can recruit both populations of lymphoid cells to lyse tumor targets. Secondly, the epitope of the T11 molecule whose expression is required for activation is not expressed in the resting, non-activated state, unlike TCR-T3. This so-called "T11$_3$" epitope is only expressed on T11-positive cells after activation with mitogen, antigen, lymphokines, or other antibodies, such as anti-T11$_2$ (Meuer S.C., et al., Cell 36:897-906 (1984)), anti-T3 and anti-2H1.

The novel bifunctional antibody constructs according to the present invention, can be used to direct only activated lymphocytes toward target cells such as tumor cells. This is achieved by binding, for example, tumor cells, in vitro or in vivo, to lymphocyte-specific/tumor cell-specific bifunctional antibody constructs wherein the lymphocyte specific component is an antibody (or one binding site of an antibody, if

the c nstruct is a hybrid antibody) specific for activated lymphocytes. Such antibodies includ , for example, the anti-T11$_3$ antibody, the anti-TAC antibodies that are specific for the IL2 receptor, the anti-Ta$_1$ antibody, th anti-T10 antibody that is specific for th T10 mol cule and anti-transf rrin rec ptor antibodies specific for the transferrin receptor, all of which are expressed preferentially on activated cells (Meuer, S.C. et al., C ll 36:897-906 (1984); Fox, D.N. et al., J. Imm 133:1250-1256 (1984); H rcendt. T. et al., Hum. Immunol. 3:247-259 (1981); and Robb, R.J. et al., J. Exp. Med. 160:1126-1146 (1984)). In this approach, th lymphocyte-specific/target cell-specific antibody construct will bind to the target tumor cells in vitro or in vivo after infusion into the body. After the antibody construct has bound to the target cells, lymphocytes can be activated in vivo or ex vivo in a variety of ways, but preferably by treatment of the lymphocytes with the combination of antibodies anti-T11$_2$ and anti-T11$_3$ which activate both T cells and NK cells. These activated lymphocytes then "home-in" on the target cells coated with the antibody construct having an anti-lymphocyte antibody or single antibody binding site component specific for the activated lymphocytes, bind to the antibody construct which brings the lymphocytes in contact with the target cells, and the bound activated lymphocytes lyse the target cells.

In this application, the production of a number of antibody constructs of anti-T11$_3$ with antitumor antibodies is disclosed. It has now been discovered that these constructs cause activated T11-expressing cells to kill tumor target cells. Furthermore, these constructs activate T11-expressing cells to kill tumor target cells when the T11-expressing cells can also bind anti-T11$_2$. Also, the production of constructs of anti-T11$_2$ with antitumor antibodies is disclosed. it has been discovered that these antibody constructs activate T11-expressing cells to kill tumor target cells when the T11-expressing cells also bind anti-T11$_3$.

The Bifunctional Antibody Constructs And Their Synthesis

Three distinct bifunctional antibody constructs are provided by the present invention.
These are:
(1) heterodimeric antibody conjugates;
(2) antibody/cell surface-binding molecule conjugates; and
(3) hybrid antibodies.

The term "bifunctional" is used to indicate that the constructs each have two different binding specificities. The term does not relate to the actual number of binding sites since, for example, a heterodimeric antibody conjugate would normally have four binding sites – two specific for the target cells and two specific for the activated lymphocytes. In contrast, a hybrid antibody has only two binding sites – one specific for the target cells and one specific for activated lymphocytes.

More specifically, according to the present invention, the bifunctional heterodimeric antibody conjugates comprise two antibodies joined together, one antibody being specific to the target cells and the other antibody being specific to activated lymphocytes.

The bifunctional antibody/cell surface-binding molecule conjugates comprise one antibody and one molecule, other than an antibody. The antibody component is specific to activated lymphocytes, while the non-antibody molecule such as, for example, hormones, lymphokines, oligosaccharides, etc. binds to target cell surface molecules.

The bifunctional hybrid antibody is one antibody having antigen binding sites to two different antigens. According to the present invention, one antigen binding site is specific for the target cells and the other is specific for activated lymphocytes.

The term "target cell" as used herein is defined as any cell, including tumor cells or other diseased cells e.g. virus infected cells, parasite infected cells, etc., which are to be killed or lysed.

Specific examples of target cells which can be employed include tumor cells, especially solid tumor cells, virus infected cells, parasite infected cells, autoimmune cells, i.e. cells which produce autoantibodies, and activated cells, i.e. those which are involved in graft rejection or graft vs. host disease.

The term "lymphocyte" as used herein is defined as a mononuclear cell which circulates in the blood and is found in lymph nodes, spleen and the lymphatics, which has a nucleus that has densely packed chromatin and a small rim of cytoplasm on staining with Wright's stain, and which has potential for cytolytic function, i.e. can lyse target cells.

Examples of suitable lymphocytes include CTL (CD8+ or CD4+), NK cells (CD2+), and T helper cells (CD4+).

Also, the term "activated lymphocyte" as used herein means a lymphocyte whose cytolytic function has been turned on by various means, some of which are described below.

The antibodies specific to the target cells can be either polyclonal or monoclonal, and are of any

isotype: IgM, IgG, IgG$_{2a}$, IgG$_{2b}$, IgG$_3$, IgG$_4$, IgA, IgE and IgD. Further the antibodies should b of human, mouse, rat, hamster or any other mammalian species origin.

The antibodi s specific to the target cells can be produced and purified according to conventional methods as follows.

First, mice, rats, hamsters, or any other mammal, including humans are immunized with the antigen of interest, such as the intact target cell, antigens isolated from the target cell, whole virus, attenuated whole virus and viral proteins such as viral coat proteins. In most cases, the entire target cell is used for immunization. The antigens used for immunization are isolated and purified by conventional techniques, see, e.g., R.I. Mishell in "Selected Methods in Cellular Immunology" (B.B. Mishell & S.M. Shiigi, eds.) San Francisco, (1980) pp. 28-65. Alternatively, pre-immune humans are identified by a serologic screen. Those who are serologically demonstrated to produce the antibodies of interest, e.g., anti-lymphocyte antibodies, such as patients with systemic lupus erythematosus or other autoimmune diseases or those who produc anti-tumor antibodies are selected.

Next, a source of B-cells (spleen, peripheral blood, lymph nodes, etc.) is taken from the immunized animals or pre-immune humans and fused with either a murine myeloma or a suitable human myeloma, such as NS-1 (P3/NS1/1-Ag4-1) (Kohler, G. et al., Eur. J. Imm. 6:292-295 (1976) – (ATCC No. TIB 18)) or SP2/0 (SP2/O-Ag14) (M. Shulman et al., Nature 276:269-270 (1978) – (ATCC No. CRL 1581)) by conventional techniques such as, for example, the polyethylene glycol (PEG) technique, e.g., according to Oi and Hertzenberg in "Selected Methods In Cellular Immunology" (Mishell, B.B. & Shilgi S.M. eds.) San Francisco, (1980) pp. 351-368. The fused cells are then cultured, screened, and cloned, all according to conventional techniques, such as described in, for example, Oi and Hertzenberg, mentioned above, to giv an immortalized clone producing the antibody of choice.

The conventional technique of in vitro immunization can also be used (R.A. Luben et al., Mol. Immun l. 17:635-639 (1980) and Hengartner H. et al., Curr. Top. Microbiol. Immunol. 81 :92-99 (1978).

After isolating the clones of interest, the monoclonal antibodies can be produced by hybridoma cells grown as ascites tumors in Balb/c or nude mice, or grown in vitro by using conventional large scale cell culture techniques. Antibodies can be purified from ascites fluid using a variety of methods including ammonium sulfate precipitation, ion-exchange chromatography and gel filtration (Parham et al., J. Immunol. Methods 53:133-173 (1982)), ion-exchange chromatography on resins coupled to Cibacron blue dye (Bruck et al, J. Immunol. Methods 53:313-319 (1982) and Bruck et al., Methods in Enzymology 121:587-596 (1986)), chromatography on columns of hydroxylapatite (Stanker et al., J. Immunol. Methods 76:157-169 (1985) and Juarez-Salinas et al., Methods in Enzymol. 121:615-622 (1986)) and affinity chromatography using immobilized protein A from Staphylococcus aureus (Goding, J. Immunol. Methods 39:285-308 (1980)). The purity of the antibody can be estimated by isoelectrofocusing gel electrophoresis (Goldmacher et al., J. Immunol. 136:320-325 (1986)).

Specific examples of antibodies that bind to target cells are described in Example 1 below.

Production and purification of polyclonal antibodies specific to the target cells can be carried out according to conventional techniques known in the art.

The antibodies specific to the activated lymphocytes can be either polyclonal or monoclonal, and are of any isotype as described above for the antibodies specific to the target cells. Further, as for the antibodi s specific to the target cells, the antibodies specific to the activated lymphocytes should be of mammalian species origin.

The antibodies specific to the activated lymphocytes can be produced and purified as follows.

The antibodies are produced by immunizing mice with human lymphoid cells and by producing immortalized antibody producing clones as described above for producing antibodies to target cells. The human lymphoid cells are obtained from the peripheral blood of any suitable human donor. Clones of interest are identified by, e.g., FACS (Fluorescent-Activated Cell Sorter) analysis of supernatants of clones on relevant cells. The clones are then grown in the appropriate mouse strain and large quantities of th antibodies can be produced from hybridoma cells grown as malignant ascites tumors. The antibody can also be produced in vitro in large quantities by standard methods, such as growing the hybridoma cell in hollow-fiber apparatus or roller bottles. These are well-known commercial processes and the necessary equipment can be obtained commercially along with appropriate instructions for use.

The monoclonal antibodies can be purified by the methods described above.

Specific examples of two such antibodies, i.e. anti-T11$_3$ and anti-T11$_2$ are described in Example 1 below.

Production and purification of polyclonal antibodies specific to the activated lymphocytes can be carried out according to conventional techniques known in the art.

Further, several suitable antibodies specific to activated lymphocytes already xist and are publicly

available including the hybridomas that produce anti-T11$_2$ (murine IgG$_{2a}$) and anti-T11$_3$ (murine IgG$_3$) which ar monoclonal antibodi s that bind to differ nt epitopes of the T11 molecule found on th surface of mature T cells (Meu r, S.C., t al., Cell 38:897-906 (1984)); anti-transferrin receptor antibody; and anti-TAC antibody. Anti-T11$_2$ and anti-T11$_3$ are commercially available from ImmunoGen Inc., Cambridge, MA. Anti

5 transferrin receptor antibody is available from the American Type Culture Collection, Rockville, MD (ATCC No. HB21). A hybridoma that produces anti-TAC⁻ antibodies is commercially available from Becton Dickins n. Mountain View, CA.

Anti-T11$_3$ is preferred as an anti-lymphocyte antibody.

These publically available antibodies can be produced (if supplied by means of a hybridoma) and
10 purified (if necessary) by the methods described above.

In order to prepare the heterodimeric antibody conjugates of the present invention, the two different antibodies may be joined together by using any of the conventional procedures for chemical cross-linking of proteins such as described by Peters et al., Annual Review Biochem. 46:523-551 (1977); Ji. Methods Enzymol. 91:580-609 (1983); Blattler et al., Biochemistry 24:1517-1524 (1985), and references therein.

15 Many of these cross-linking reagents are available commercially, for example from Pierce Chemical Company, Rockford, IL, Sigma Chem. Co., St. Louis, MO, Boehringer Mannheim Biochemicals, Indianapolis, IN and Pharmacia, Uppsala, Sweden. Preferably, the two different antibodies are modified with heterobifunctional cross-linking reagents which will introduce complimentary reactive groups, for example maleimido groups and sulfhydryl groups, and then the different modified antibodies are subsequently cross-linked by a
20 reaction of the complimentary groups that yields only the heterodimer in high yield, as illustrated in Scheme 1 below.

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SCHEME 1

1

2

2-iminothiolane

SMCC

SH

O

N

O

MIX

1

O

S

N

O

2
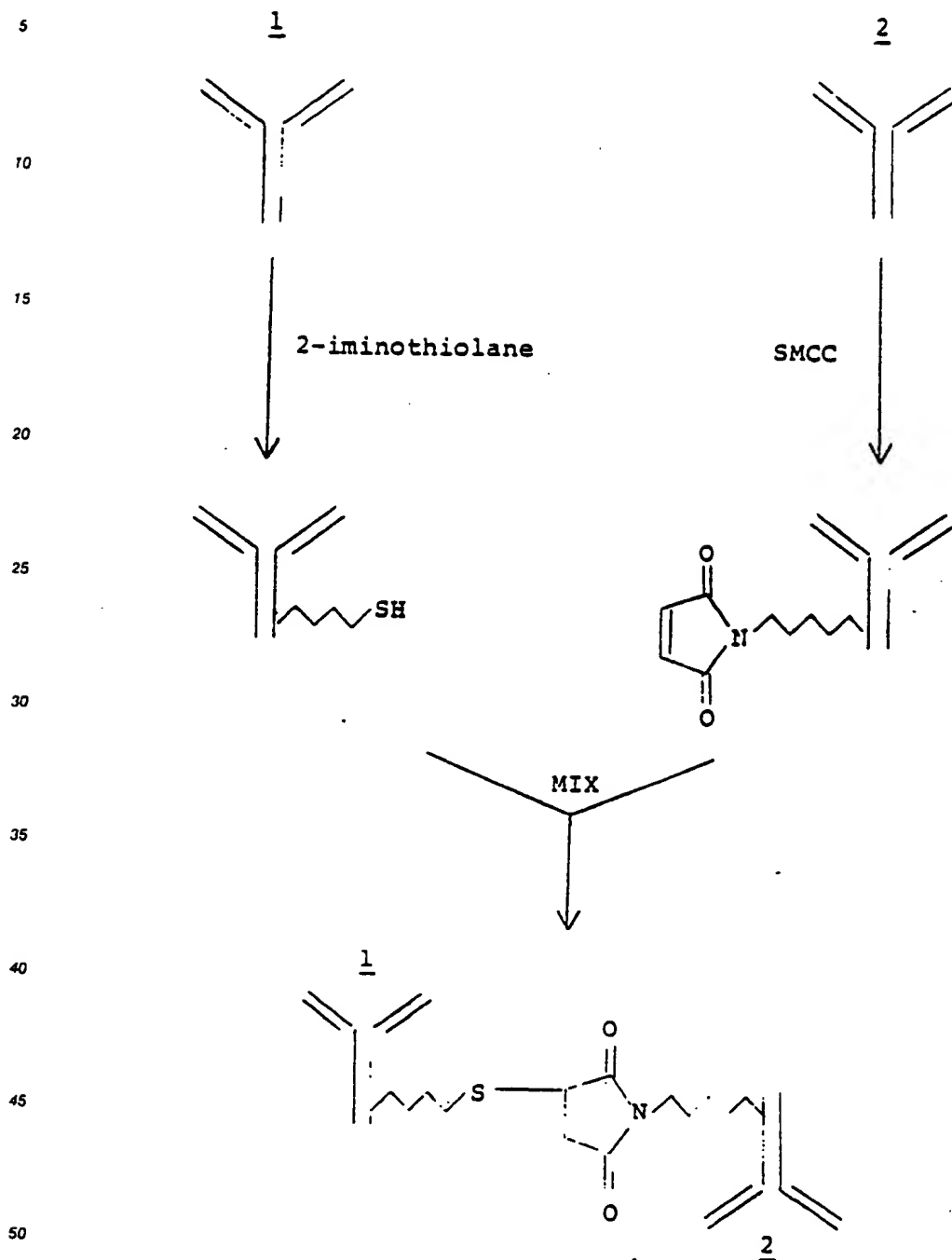
In Scheme 1, antibody 1 is modified with 2-iminothiolane which reacts with amino groups found on all proteins to introduce sulfhydryl groups into the antibody (Traut et al., Biochemistry 12:3266-3275 (1973); Lambert et al., Biochemistry 17:5406-5416 (1978)). Sulfhydryl groups may be introduced into antibodies using a number of other reagents, such as N-succinimidyl 3-(2-pyridyldithio)propionate (Carlsson et al., Biochem. J. 173: 723-737 (1978)), and by mild reduction with reducing agents by, e.g. treating antibodies with 2-mercaptoethanol (2-20 mM) or dithiothreitol (1-2 mM) for a short tim (15-40 min) at 4° C or ambi nt

t mperature. followed by gel filtration or dialysis to remov excess reducing agent.

In Scheme 1, antibody 2 is modified with succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) which reacts with amino groups found on all proteins to introduce maleimido groups into the antibody (.g., Lambert t al., J. Biol. Chem. 260:12035-12041 (1985)). Mal imido groups may also be introduced into proteins using other het robifunctional reagents, f r example m-maleimidobenzoyl-N-hydroxysuccinimide ster and succinimidyl 4(p-maleimidophenyl)butyrate are two compounds available commercially from Pierce Chemical Co., Rockford, IL, maleimidohexanoyl-N-hydroxysuccinimid ester is available commercially from Boehringer Mannheim Biochemicals, Indianapolis, IN, and see Blattler et al., Biochemistry 24:1517-1524 (1985). Other groups, for example α-chloro-, α-bromo-or α-iodo-carbonyl func-tions, are also reactive with sulfhydryl groups and such groups can be introduced into antibody 2 instead of maleimido groups to enable antibody 1 and antibody 2 to be efficiently cross-linked. For example, antibody 2 can be modified with N-succinimidyl iodoacetate to introduce iodoacetyl groups into the antibody. 2-Pyridyldithio groups are also reactive with free sulfhydryl groups and such groups can also be introduced into antibody 2 by reaction with N-succinimidyl 3-(2-pyridyldithio) propionate, for example. In this case, the cross-linking reaction between antibody 1 and antibody 2 will result in a cross-link containing a disulfide bond (see Lambert et al., J. Biol. Chem. 260: 12035-12041 (1985)).

Sulfhydryl groups can be introduced into either one of antibody 1 or antibody 2, and groups that will react with sulfhydryl groups such as maleimido groups can be introduced into either one of antibody 2 or antibody 1 for efficient formation of a cross-link between antibody 1 and antibody 2.

Sulfhydryl groups may be introduced into both antibodies, which then may be oxidized to form disulfide bonds, some of which can link antibody 1 and antibody 2. Sulfhydryl groups introduced into both antibodies may also be cross-linked by bifunctional alkyl dihalides or by bifunctional bis-maleimides such as N,N-O-phenylenedimaleimide and bismaleimidohexane.

Another method by which an antibody 1 - antibody 2 cross-link can be formed is to conjugate one of the antibodies to biotin using one of the commercially available derivatives of biotin that will react with proteins such as N-hydroxysuccinimidobiotin or sulfosuccinimidyl 6-(biotin-amido) hexanoate (Pierce Chemical Co., Rockford, IL), and to conjugate the second antibody to avidin or to streptavidin using any of the cross-linking procedures for joining two proteins that have been summarized above, or see Lambert et al., J. Boil. Chem. 260:12035-12041 (1985), for example. Avidin and streptavidin bind to biotin, and thus antibody 1 containing biotin and antibody 2 cross-linked to avidin or to streptavidin will form complexes containing both antibodies, upon mixing the two thusly-modified antibodies together.

The antibody/cell surface-binding molecule conjugate according to the present invention can be produced according to the methods described above for producing the heterodimeric antibody conjugate except that the antibody specific to the target cell is replaced with a molecule, other than an antibody, which can bind to cell surface molecules.

Examples of suitable molecules which can be used in the antibody/cell surface-binding molecule conjugate include hormones, e.g. insulin, TRH (thyrotropin releasing hormones), MSH (melanocyte stimulating hormone); lymphokines, e.g. IL-2, IL-3, interferons (e.g. α, γ, β); and colony stimulating factors (CSF's), e.g. G-CSF, GM-CSF; etc.

Hybrid antibodies containing binding sites having different specificities according to the present invention can be produced by several conventional methods.

(1) Two antibody producing myelomas can be fused to form a hybridoma according to the method of Cotton and Milstein (Nature 244:42-43 (1973)).

(2) Hybridoma cells producing a monoclonal antibody can be fused with spleen cells of a mouse which has been immunized with the appropriate antigen (see, e.g., K.S. Webb et al., Canc. Treat. Rep. 69:663 (1985)).

(3) Two hybridoma cells, each of which produces a monoclonal antibody can be fused (see, e.g., Stearz and Bevan, PNAS 83: 1453-1457 (1986)).

Fusions according to methods (1), (2) or (3) are typically performed according to Oi and Hertzenberg in "Selected Methods in Cellular Immunology" (Mishell B., Shiigi S. eds.) Oxford 1980, pp. 351-368.

(4) Genetic engineering can be used to introduce a gene of another heavy chain and light chain into an antibody producing hybridoma cell as described, e.g., in S.D. Gillies et al. Cell 33:717-728 (1983).

The thus synthesized bifunctional antibody constructs are purified for further use as follows.

Heterodimeric antibody conjugates and antibody/cell surface-binding molecule conjugates can be purified by gel filtration. Other conventional procedures can also be used including, in particular, hydroxylapatite chromatography (Juarez-Salinas et al, Meth. Enzymology 121:615-622 (1986).

Example 3 below describes in detail a method for purifying specific heterodimeric antibody conjugates. Th meth d can be appli d to the purification of any such conjugat .

The hybrid antibodies can b purified by the methods described above for purifying monoclonal antibodi s along with mploying hydroxylapatit chromatagraphy referred to above.

The ability of the heterodimeric antibody conjugates to bind to respective cells can be evaluated as follows.

Cell lines which xpress on the surface antigen for one component of the heterodim r and not for the other component are used. Cells are incubated with the dimer at concentration X, for example, 50 μg/ml, or with free antibody at concentration 1/2 X, for example 25 μg/ml, for about 30 min. on ice; then the cells are washed twice and stained with an appropriate dilution of goat anti-mouse or rabbit anti-mouse immunoglobulin conjugated to a suitable label such as fluorescein isothiocyanate (FITC) for about 30 min. on ice. The cells are next washed again and assayed for fluorescence intensity on a cytofluorograph. Heterodimeric antibody conjugate, if fully functional, will give in this assay the same fluorescence intensity distribution as free antibody, thus indicating that the conjugate binds equally well to the relevant antigen on the cell.

The ability of antibody/cell surface-binding molecule conjugates and hybrid antibodies to bind to respective cells can be evaluated by similar conventional techniques well known to the skilled artisan.

Activation of Lymphocytes

Lymphocytes can either be activated in vivo or ex vivo. If treatment of the target cells is in vivo and the lymphocytes are activated ex vivo, the lymphocytes are infused back into the body after activation.

In vivo activation fo lymphocytes is achieved by infusion of activating antibodies, such as anti-T11₂, anti-T3 (available from Ortho Pharmaceuticals, Raritan, NJ by the name OKT-3), or anti-2H1 several hours prior to infusion of the bifunctional antibody construct.

In vivo activation of lymphocytes can be achieved by:

(1) inducing the expression of T11₃ by infusion of anti-T11₂, anti-2H1 (an anti-2H1 producing hybridoma has been deposited with the American Type Culture Collection, Rockville, MD., Deposit No. ATCC HB 9399), or anti-T3; or

(2) inducing the expression of T11₃ as described above and activation by lymphokines, such as IL-2, BSF-1, and γ-IFN.

Preferred substances include the activating antibodies anti-T11₂, anti-T3 and anti-2H1.

Ex vivo activation of lymphocytes can be carried out for several days prior to infusion of the bifunctional antibody construct.

Ex vivo activation of lymphocytes is achieved by incubation of peripheral blood lymphocytes (PBL's) removed from blood by cytophoresis using conventional medical equipment with IL-2, anti-T11₂, anti-T3 or anti-2H1. Incubation is performed at 0.5 x 10⁶ cells/ml with soluble anti-T11₂ or anti-2H1 at, e.g., 10 μg/ml (1 μg/ml to 100 μg/ml), or IL-2 at, e.g., 10-1000 U/ml, or anti-T3 adhered to plates or bound to beads at, e.g., 10 μg/ml (1 μg/ml to 100 μg/ml) in, e.g., RPMI 1640 medium supplemented with 10% human AB serum at 37°C in an atmosphere of 100% humidity and 5-10% CO₂.

Substances that can be used to activate lymphocytes ex vivo include IL-2, anti-T11₂, anti-T3, and anti-2H1.

Preferred substances include anti-T11₂, anti-T₃, and anti-2H1, and especially preferred is anti-T11₂

The ability of substances to activate lymphocytes in vivo can be determined by infusing into the patient the activating substance, such as the antibodies mentioned above, then withdrawing blood, isolating the PBLs and assaying, e.g., for T11₃ expression and killing ability with the appropriate corresponding bifunctional antibody construct. Specific conditions of the assay can readily be determined by the skilled artisan.

The ability of substances to activate lymphocytes ex vivo can be determined by, e.g., the ability of th substances to generate killer cells after 3 days in vitro culture with the above-described activating reagents. Killer cell function is measured by monitoring the ability of activated PBL's to kill targets using one of the bifunctional antibody constructs of the present invention or by monitoring lectin mediated lysis in accordance with conventional methods.

The present invention is further illustrated by reference to a preferred embodiment but the present invention is not to be construed as being limited thereby.

In this preferred embodiment of the present invention, the heterodimeric antibody conjugate contains anti-T11₃ as the anti-lymphocyte antibody component and an anti-tumor cell antibody as the anti-target cell antibody component. According to this embodiment, the anti-T11₃/anti-tumor cell antibody conjugate can be infused prior to th infusion of activated cells without concern that the conjugate will bind to the huge

number of non-activated T11-expr ssing cells that circulate in vivo. Thus, th very low expr ssion of th T11₃ epitope on these circulating normal n n-activated T cells allows the anti-T11₃-antitum r cell antibody conjugate to bind efficiently to the target tumor cells in vivo. When activated lymphocytes are infused subsequently, the activated lymphocytes targ t specifically the tumor cells that are coated with the anti-
5 T11₃-antitumor cell antibody conjugate.

For example, in on approach, T cells can be treated ex vivo with anti-T11₂, which will expose th anti-T11₃ epitope on the surface of th T cells. Upon infusion back into the body f the host, the anti-T11₂-coated cells bind to the tumor cells coated with anti-T11₃ heterodimeric antibody conjugate. The T cells are thus activated by the binding of anti-T11₂ and anti-T11₃ and are in close cell-cell contact with the target
10 tumor cells by the heterodimeric antibody conjugate bridge that enables the activated lymphoid cells to kill the target cells.

In Example 5, set forth below, target cells were coated with a heterodimeric antibody conjugate of anti-T11₃ and an antitumor antibody specific for the target cells. When these coated target cells were mixed with activated T11-expressing cells, the target cells and the T11-expressing cells were bound together in
15 close cell-cell contact by the heterodimeric antibody conjugate which formed a bridge between the cells. The close cell-cell contact that results from the antibody bridge allowed the activated T11-expressing cells to lyse the target cells.

20 ## Method of In Vivo Treatment

According to the present invention, the bifunctional antibody constructs can be delivered to the patient as an intravenous infusion in normal saline with normal human albumin added as protein carrier.

The amount of bifunctional antibody construct administered varies with age, body weight and tumor
25 burden, but generally is in the range of 0.1 to 10 mg/kg of body weight.

Intravenous IL-2 has been shown to activate killer cells in vivo (S.A. Rosenberg at al, J. Exp. Med. 161:1169-1188 (1985)), and this method can be used to obtain activated cytolytic lymphocytes. In vivo activation of lymphocytes should also proceed as described for IL-2 for the reagents described above in the section headed "Activation of Lymphocytes".

30 For ex vivo activation of lymphocytes, lymphocytes are derived from the patient as described above and activated as described above. The activated lymphocytes are then returned to the body by continuous intravenous infusion at a suitable rate, such as at a rate of 10⁴ cells/hour in a volume of 50 ml/hr.

Examples of medical conditions that can be treated according to the in vivo method include malignancy of any type including, for example, cancer of the lung, breast, colon, prostate, kidney and lymphatic organs;
35 autoimmune diseases, such as systemic lupus, rheumatoid arthritis, and multiple sclerosis; graft rejections, such as renal transplant rejection, cardiac transplant rejection, liver transplant rejection, lung transplant rejection, and bone marrow transplant rejection; graft vs. host disease; viral infections, such as CMV infection, HTLV infection, AIDS, etc.; and parasite infections, such as giardiasis, amoebiasis, shistosomiasis, etc.

40

Method of In Vitro Treatment

The method of the present invention can also be performed in vitro.
45 Examples of medical conditions that can be treated according to the in vitro method include bone marrow transplants, autologous or allogeneic.

In vitro treatment can be carried out as follows.

PBL are harvested from the patient, activated as described above (with IL-2, or/and anti-T11₂, anti-T3 anti-2H1) for several days. Bone marrow, which contains cells to be targeted, e.g. tumor cells, are mixed
50 with the activated cells at E/T (effector cell/target cell) ratios of 20:1 to 1:1 and the appropriate bifunctional antibody construct for 4 to 18 hours. After this time, the bone marrow cells are washed with medium containing serum and returned to the patient by i.v. infusion according to known methods.

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EXAMPLES

Th present inv ntion will now be described by ref rence to specific exampl s, which are not meant to
be limiting.

Unless otherwise specified, all percents, ratios, etc. are by weight.

Monoclonal Antibodies Described in the Examples.

The hybridoma cell lines that produce antibodies anti-T11₂, anti-T11₃, J5 and anti-B4 were produced as disclosed in the cited references.

Anti-T11₂ and anti-T11₃ are monoclonal antibodies that bind to different epitopes of the T11 molecule found on the surface of mature T cells (Meuer S.C., et al., Cell 36:897-906 (1984)).

J5 is a murine IgG₂ₐ that is specific for the Common Acute Lymphoblastic Leukemia Antigen (CALLA) (Ritz J., et al., Nature 283:583-585 (1980)).

Anti-B4 is a murine IgG₁ that is specific for the B4 antigen found on B cells and malignancies of B cell origin (Nadler L.M., et al., J. Immunol. 131:244-250 (1983)).

The hybridoma cell lines producing these antibodies were grown as ascites tumors in Balb/C mice. Alternatively, J5 and anti-B4 can be obtained commercially from Coulter Immunology, Hialeah, FL. Anti-T11₂ and anti-T11₃ antibodies can be obtained commercially from ImmunoGen Inc., Cambridge, MA.

EXAMPLE 1

Purification of Antibodies

All purification steps were performed at 4°C. The J5 antibody was purified from ascites fluid using conventional methods (Goldmacher et al., J. Immunol. 136:320-325 (1986); Lambert et al., J. Biol. Chem. 260:12035-12041 (1985)) using affinity chromatography on a column of protein A-Sepharose CL-4B (Sigma Chemical Co., St. Louis, Mo.). The antibody bound by the column was eluted with 0.1 M acetic acid containing NaCl (0.15 M). Antibody-containing fractions were immediately neutralized by the addition of one-tenth volume of 1.0 M NaHCO₃, and were then dialysed against 10 mM sodium phosphate buffer, pH 6.0, containing glycine (50 mM) and NaN₃ (0.4 mM), for further purification by ion-exchange chromatography on a column of CM-cellulose (CM-52; Whatman Chemical Separations, Clifton, N.J.) equilibrated in the same buffer. The column (30 ml bed volume for 120 mg of protein) was developed with a linear gradient of NaCl (0 to 100 mM), and J5 eluted as a single peak. The purified antibody was finally dialysed against 10 mM potassium phosphate buffer, pH 7.2, containing NaCl (145 mM) and then sterilized by filtration (0.22 μm Millex-GV membrane, Millipore Corp., Bedford, MA or 0.2 μm Uniflo membranes, Schleicher & Schuell, Keene, NH) and stored at a concentration of about 5 mg/ml at -70°C.

Anti-T11₂ was purified by a similar procedure, except that the CM-cellulose columns used in th second step of purification of this antibody was developed with a linear gradient of 0 to 300 mM NaCl in th 10 mM sodium phosphate buffer, pH 6.0, containing glycine (50 mM) and NaN₃ (0.4 mM).

. Anti-B4 was purified in three steps at 4°C. First, ascites fluid was diluted with 2 volumes of 10 mM potassium phosphate buffer, pH 7.2, containing NaCl (145 mM) and then solid (NH₄)₂SO₄ was added in small portions to the continuously stirred solution over 45 min. The suspension was stirred for 1 hour. Th precipitated protein was recovered by centrifugation and then dissolved (in 50 ml of protein from 50 ml of ascites fluid) in 0.1 M Tris.HCl buffer, pH 8.9, containing NaCl (3.0 M) and dialysed against 100 vol. of th pH 8.9 buffer for 8 h. The dialysed solution was applied to an affinity column of protein A-Sepharose CL-4B that was equilibrated in the pH 8.9 buffer, and then the column was washed with pH 8.9 buffer until th absorbance of the eluate was near zero. The anti-B4 antibody was then eluted from the column with 50 mM sodium phosphate buffer, pH 6.0, containing sodium acetate (50 mM) and NaCl (150 mM). The antibody-containing fractions were concentrated for final purification by gel filtration through a column of Sepharose S-300 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated in 10 mM potassium phosphate buffer, pH 7.2, containing NaCl (145 mM). The purified antibody was sterilized and stored as described for J5 (above).

Anti-T11$_3$ was purified by amm nium sulfate precipitation and affinity chromatography on protein A-Sepharose CL-4B using the same procedur as for anti-B4, except that the antibody was eluted from th pr tein A-Sepharose CL-4B column with 0.1 M acetic acid containing NaCl (0.15 M), collecting fractions dir ctly into tubes containing 1.0 M NaHCO$_3$ (one-tenth fracti n volume). The antibody-containing fractions w re combined and dialysed exhaustively against 40 mM potassium phosphate buffer, pH 7.2, containing NaCl (0.58 M). The purified antibody was sterilized by filtration and stored at a concentration of about 5 mg/ml at -70 $^\bullet$ C.

EXAMPLE 2

Chemical Conjugation of J5 or Anti-B4 to Anti-T11$_2$ or Anti-T11$_3$

The J5 antibody (2 mg/ml) in 60 mM triethanolamine.HCl buffer, pH 8.0, containing EDTA (1 mM) and NaCl (100 mM) was degassed and then treated with 2-iminothiolane (0.13 mM) for 90 min at 0 $^\bullet$ C under a nitrogen atmosphere. Stock solutions of 2-iminothiolane (Sigma Chemical Co., St. Louis, MO) were prepared as described by Lambert et al., Biochemistry, 17:5406-5416 (1978). The reaction was terminated by gel filtration at 4 $^\bullet$ C through a column of Sephadex G-25 (superfine) equilibrated with 5 mM bistris.acetate buffer, pH 5.8, containing NaCl (50 mM) and EDTA (1 mM). Sulfhydryl groups introduced into J5 antibody in this way were quantified spectrophotometrically by the method of Ellman, Arch. Biochem. Biophys. 28:70-77 (1959): about 0.7 sulfhydryl groups per molecule of J5 were introduced by the procedure described here.

Anti-B4 antibody was modified by exactly the same procedure as J5 antibody except that the 2-iminothiolane concentration used in the 90 min incubation at 0 $^\bullet$ C was 0.26 mM.

Maleimido groups were introduced into the antibodies anti-T11$_2$ and anti-T11$_3$ using the same method for each. The antibodies (1 mg/ml) in 100 mM sodium phosphate buffer, pH 7.0, containing EDTA (1 mM) were incubated with succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Pierce Chemical Co., Rockford, IL) for 30 min at 30 $^\bullet$ C using a reagent concentration for each antibody that resulted in the introduction of 1.0 maleimido group per molecule: 20 μM for anti-T11$_2$ and 10 μM for anti-T11$_3$. The reagent was added from a freshly prepared 10 mM stock solution in dry dioxane. Reactions were terminated by gel filtration at 4 $^\bullet$ C through columns of Sephadex G-25 (superfine) equilibrated with the pH 7.0 buffer. The level of incorporation of maleimido groups was measured by an assay using radiolabelled cysteine as described by Lambert et al., J. Biol. Chem. 260:12035-12041 (1985).

J5 or anti-B4 (0.5 to 1.0 mg/ml) containing introduced sulfhydryl groups, in 5 mM bistris. acetate buffer, pH 5.8, containing NaCl (50 mM) and EDTA (1 mM), was mixed with an equal weight of an antibody (0.5 to 1.0 mg/ml of anti-T11$_2$ or anti-T11$_3$) containing introduced maleimido groups in 100 mM sodium phosphate buffer, pH 7.0, containing EDTA (1 mM). The pH of the mixture was adjusted to 7.0 by the addition of 0.5 M triethanolamine.HCl, pH 8.0, and the mixture was then incubated under nitrogen for 16 hours at 4 $^\bullet$ C. Then, 2-mercaptoethanol was added to a final concentration of 2.5 μM to block any remaining maleimido groups, and, after 30 min incubation at 4 $^\bullet$ C, iodoacetamide (2 mM) was added and incubation was continued for another 60 min in order to block all free sulfhydryl groups.

EXAMPLE 3

Purification of Heterodimeric Antibody Conjugates

The conjugation reaction mixture was subjected to gel filtration through a column (95 cm x 2.6 cm for 10 ml sample volume) of Bio-Gel A-1.5 m (fine) (Bio-Rad, Richmond, CA) equilibrated in 10 mM HEPES.NaOH buffer, pH 7.6, containing NaCl (3M). Under these conditions, antibody-antibody dimers ($\underline{M}_r$ = 320,000) were completely resolved from aggregates of high $\underline{M}_r$ and from monomeric IgG antibodies (($\underline{M}_r$ = 160,000). Samples from fractions eluting from the column w re analyzed by polyacrylamide/dodecyl sulfate

gel electrophor sis in gel slabs (145 mm x 90 mm x 0.75 mm) cast with acrylamide gradients (5-10% w/v). Gels were run under non-reducing conditions using th procedure f Laemmll, Nature 227:680-685 (1970); Lambert t al., J. Biol. Chem. 260:12035-12041 (1985). Fractions containing purified antibody-antibody heterodimer were pooled, concentrated to about 0.5 mg/ml using a CX-30 immersibl membrane (Millipore Corp., Bedford, MA), and finally dialysed into 10 mM potassium phosphat buffer, pH 7.2, containing NaCl (145 mM). Samples were stored frozen at -70° C.

EXAMPLE 4

Evaluation of Binding for the Heterodimeric Antibody Conjugates: Analysis by Fluorescence-activated Cell Sorting

The ability of the J5-anti-T11$_3$ and J5-anti-T11$_2$ conjugates to bind to CALLA-expressing and T 11$_2$/T11$_3$-expressing cells was evaluated. The cell line used was Namalwa, a CALLA+, B4+, T11- Burkitt lymphoma line available from the American Type Culture Collection, Rockville, MD and having ATCC No. CRL 1432 (Int. J. Cancer 12:396-408 (1973)) and REX, a T11$^•$ CALLA +/- adult T-cell leukemia cell line as an example of a T11$_3$ and T11$_2$ positive cell line. However, any such cell line or also activated peripheral blood lymphocytes from any healthy human donor can be used in place of REX.

Conjugates, or the equivalent amount of unmodified antibody (i.e., J5, anti-B4, anti-T11$_2$, or anti-T11$_3$) were incubated with 1 x 10^6 cells in a volume of 50 μl for 30 minutes on ice, were washed twice with 1 ml of Hanks Balanced Salt Solution (HBSS) containing 1% bovine serum albumin (BSA). The cells were then incubated with a 1:25 dilution of goat-anti-mouse Ig fluorescein isothiocyanate (GAMG-FITC) in a volume of 25 μl on ice, washed twice with HBSS.1% BSA, and finally the cells were analyzed for fluorescence on a Fluorescence-Activated Cell Sorter (Epics IV). The Figure shows the patterns obtained for J5, anti-T11$_2$, anti-T11$_3$ and conjugates J5-anti-T11$_2$ and J5-anti-T11$_3$.

More specifically, the Figure shows a FACS analysis of heterodimeric conjugates wherein Namalwa cells (groups a through h) or REX cells (groups i through p) were incubated with no antibody (a,i), J5 (b,j), anti-T11$_2$ (c,k), anti-T11$_3$ (d,l), anti-T3 (e,m), J5-anti-T11$_2$ (f,n), J5-anti-T11$_3$ (g,o) or J5-anti-T3 (h,p).

It is evident from the Figure that each antibody component in the heterodimeric conjugate has retained the same binding activity and specificity as the free antibody.

EXAMPLE 5

Activation of Peripheral Blood Lymphocytes (PBL) by anti-T11$_2$/anti-T11$_3$ Containing Conjugates

In addition to the ability to bind cells expressing relevant antigens, and anti-T11$_2$/anti-T11$_3$ conjugates must be shown to have functional properties, i.e., must activate T11 + PBL under the appropriat circumstances. It is known that the combination of anti-T11$_2$ and anti-T11$_3$ antibodies can activate PBL to proliferate (Meuer S.C., et al., Cell 36:897-906 (1984)). Therefore, the functional integrity of the conjugates of this invention was assessed by incubating PBL in the presence of the J5-anti-T11$_3$ conjugate and anti-T11$_2$ or in the presence of J5-anti-T11$_2$ and anti-T11$_3$, and measuring proliferation of sheep erythrocyte-rosetted PBL by the incorporation of ^{3}H-thymidine. Specifically, 5 x 10^4 PBL were incubated for 72 hrs in flat-bottom microtitre plates in the presence of the indicated reagents as shown in Table 1 below, and wer pulsed with 1 μCl of thymidine for the last 18 hours.

The results obtained are also shown in Table 1.

Tabl 1

Activation of Human PBL by Heterodimeric Conjugates of J5 and Anti-T11$_2$ or Anti-T11$_3$	
In Vitro Addition	CPM ± S.D. [1]
–	365 ± 22
Con A [2], 2.5 μg/cc	42,408 ± 2262
Anti-T11$_2$	730 ± 177
Anti-T11$_3$	2,254 ± 1103
J5-anti-T11$_2$	650 ± 390
J5-anti-T11$_2$ + anti-T11$_3$	38,359 ± 390
J5-anti-T11$_3$	408 ± 102
J5-anti-T11$_3$ + anti-T11$_2$	30,641 ± 2,582
J5 + anti-T11$_2$ + anti-T11$_3$	25,125 ± 545

[1] Counts per minute ± standard deviation
[2] Con A = Concanavalin A

It is evident from the results in Table 1 above that both the J5-anti-T11$_2$ and J5-anti-T11$_3$ conjugates contain functional T11$_2$ and T11$_3$ antibodies, respectively, as they are able to activate PBL in the presence of the appropriate free antibody.

EXAMPLE 6

Lysis of a CALLA+ Tumor by Human CTL in the Presence of J5-anti-T11$_3$ and J5-anti-T11$_2$ Conjugates

Clone TB1-6 (received from Dr. Chikao Morimoto), a representative example of a human CTL clone, which is specific for the EBV-transformed human B cell line, CM-EBV, was used as the effector cytolytic cell in this example. The CTL clone was incubated for 4 hours with the CALLA+ tumor Namalwa, ATCC No. CRL 1432, Int. J. Cancer 12.:396-408 (1973), which the clone does not normally lyse, or in the presence of the CALLA-tumor OVCAR available from the American Type Culture Collection. ATCC No. HTB161, Canc. Res. 44:5286-5290 (1984) in the presence of a final concentration of 10 μg/ml of J5-anti-T11$_3$ or J5-anti-T11$_2$, or other reagents as shown in Table 2 below, either 1 x 10^5 effector cytolytic cells (TB1-6) or 0.5 x 10^5 T cells, and 5 x 10^3 ^{51}Cr-labelled Namalwa targets were incubated in V-bottom plates in a final volume of 200 ul in medium consisting of RPMI 1640 with 10% FCS and 100 μg/ml streptomycin and 100 U/ml penicillin. After four hours, supernatants were harvested and the percent specific lysis calculated according to standard methods (Siliciano R.F., et al., Nature 317:428-429 (1985)).

The results are also shown in Table 2.

14

Table 2

Heterodim ric Conjugates of Anti-T11$_2$ or Anti-T11$_3$ and J5 Direct the Lysis of CALLA+ Targets by a Human CTL Clone			
Target	In Vitro Addition	% Specific Release	
		20:1	10:1
CM-EBV	—	77	80
Namalwa	—	1	0
	J5-anti-T11$_3$	6	8
	J5-anti-T11$_3$ + anti-T11$_2$	36	41
	J5-anti-T11$_2$	1	1
	J5-anti-T11$_2$ + anti-T11$_3$	28	33
	J5 + anti-T11$_2$ + anti-T11$_3$	18	16
OVCAR	—	19	12
	J5-anti-T11$_2$ + anti-T11$_3$	22	18
	J5-anti-T11$_3$ + anti-T11$_2$	23	23
	J5 + anti-T11$_2$ + anti-T11$_3$	27	21

It is evident from Table 2 that either the J5-anti-T11$_3$ or J5-anti-T11$_2$ conjugate, in the presence of th reciprocal anti-T11 antibody (i.e., anti-T11$_2$ and anti-T11$_3$, respectively) can direct TBI-6 to lyse the CALLA+ tumor Namalwa, but not the CALLA- tumor, OVCAR.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

Claims

1. A bifunctional heterodimeric antibody conjugate comprising (a) an anti-target cell antibody component joined to (b) an anti-lymphocyte antibody component wherein the anti-lymphocyte antibody component is specific for activated lymphocytes.

2. The bifunctional heterodimeric antibody conjugate of Claim 1, wherein said anti-lymphocyte antibody component (b) is an anti-T11$_3$ antibody.

3. The bifunctional heterodimeric antibody conjugate of Claim 1, wherein said anti-lymphocyte antibody component (b) is an anti-T11$_2$ antibody.

4. The bifunctional heterodimeric antibody conjugate of Claim 1, wherein said anti-target cell antibody component (a) is one member selected from the group consisting of an anti-tumor cell antibody, an antibody to a virus infected cell, an antibody to a parasite infected cell, an antibody to an autoimmune cell, an antibody to activated cells that cause graft rejection and an antibody to activated cells that cause graft vs. host disease.

5. The bifunctional heterodimeric antibody conjugate of Claim 2, wherein said anti-target cell antibody component (a) is one member selected from the group consisting of an anti-tumor cell antibody, an antibody to a virus infected cell, an antibody to a parasite infected cell, an antibody of an autoimmune cell, an antibody to activated cells that cause graft rejection and an antibody to activated cells that cause graft vs. host disease.

6. The bifunctional heterodimeric antibody conjugate of Claim 1, wherein said antibody components (a) and (b) of the heterodimeric antibody conjugate are joined by cross-linked complimentary reactive groups.

7. A bifunctional antibody/cell surface-binding molecule conjugate comprising (a) a molecular component, other than an antibody, capable of binding to target cell surface molecules and (b) an anti-lymphocyte antibody component wherein the anti-lymphocyte antibody component is specific for activated lymphocytes.

8. The bifunctional antibody/cell surface-binding molecule conjugate of Claim 7, wherein said anti-lymphocyte antibody component (b) is an anti-T11$_3$ antibody.

.9. The bifunctional antibody/cell surface-binding molecule conjugate of Claim 7, wherein said anti-lymphocyte antibody compon nt (b) is an anti-T11$_2$ antibody.

10. The bifunctional antibody/cell surface-binding molecule conjugate of Claim 7, wherein said molecular component (a) is capable of binding to on member selected from the group consisting of tum r cell surface molecules, virus infected c ll surface molecules, parasite infected cell surface molecules, autoimmune cell surface molecules, surface molecules of activated cells that cause graft rejection and surface molecules of activated cells that cause graft vs. host disease.

11. The bifunctional antibody/cell surface-binding molecule conjugate of Claim 8, wherein said molecular component (a) is capable of binding to one member selected from the group consisting of tumor cell surface molecules, virus infected cell surface molecules, parasite infected cell surface molecules, autoimmune cell surface molecules, surface molecules of activated cells that cause graft rejection and surface molecules of activated cells that cause graft vs. host disease.

12. The bifunctional antibody/cell surface-binding molecule conjugate of Claim 7, wherein said components (a) and (b) of the conjugate are joined by cross-linked complementary reactive groups.

13. The bifunctional antibody/cell surface-binding molecule conjugate of Claim 7 or 8, wherein said molecular component (a) is one member selected from the group consisting of hormones, lymphokines, and colony stimulating factors.

14. A bifunctional hybrid antibody comprising (a) an anti-target cell binding site component and (b) an anti-lymphocyte binding site component, wherein the anti-lymphocyte binding site component is specific for activated lymphocytes.

15. The bifunctional hybrid antibody of Claim 14, wherein said anti-lymphocyte binding site (b) is specific for T11$_3$.

16. The bifunctional hybrid antibody of Claim 14, wherein said anti-lymphocyte binding site (b) is specific for T11$_2$.

17. The bifunctional hybrid antibody of Claim 14, wherein said anti-target cell binding site (a) is specific for one member selected from the group consisting of tumor cells, virus infected cells, parasite infected cells, autoimmune cells, activated cells that cause graft rejection and activated cells that cause graft vs. host disease.

18. The bifunctional hybrid antibody of Claim 15, wherein said anti-target cell binding site (a) is specific for one member selected from the group consisting of tumor cells, virus infected cells, parasite infected cells, autoimmune cells, activated cells that cause graft rejection and activated cells that cause graft vs. host disease.

19. A method for selectively destroying target cell populations using a bifunctional heterodimeric antibody conjugate which comprises (a) an anti-target cell antibody component joined to (b) an anti-lymphocyte antibody component, wherein said anti-lymphocyte antibody component is specific for activated lymphocytes, said method comprising:

(1) binding the bifunctional heterodimeric antibody conjugate through the component (a) to target cells;

(2) activating lymphocytes; and

(3) binding the activated lymphocytes to the component (b) of the bifunctional heterodimeric antibody conjugate;

wherein said steps (1), (2) and (3) can be conducted simultaneously or in any order, provided that step (2) is always conducted simultaneously with or prior to step (3).

20. The method of Claim 19, wherein the method comprises destroying the target cells in vitro.

21. The method of Claim 19, wherein the method comprises destroying the target cells in vivo.

22. The method of Claim 21, wherein the method comprises activating the lymphocytes in vivo or ex vivo.

23. The method of Claim 19, 20, 21 or 22, wherein the method comprises activating said lymphocytes with one member selected from the group consisting of antibodies, mitogens, antigens, and lymphokines.

24. The method of Claim 23, wherein the method comprises activating the lymphocytes with one or more antibodies.

25. The method of Claim 24, wherein the method comprises activating the lymphocytes with one or more antibodies selected from the group consisting of anti-T11$_2$, anti-T3, and anti-2H1.

26. The method of Claim 22, wherein the method comprises activating the lymphocytes with a mixture of anti-T11$_2$ and anti-T11$_3$ antibodies.

27. The method of Claim 22, wherein said anti-lymphocyte antibody component (b) of said bifunctional heterodimeric antibody conjugate is an anti-T11$_3$ antibody and said method comprises activating said lymphocytes in vivo or ex vivo with anti-T11$_2$.

28. The method of Claim 19, wh rein said anti-lymphocyte antibody component (b) of said bifunctional heterodimeric antibody conjugat is an anti-T11$_2$ component.

29. The method of Claim 19, 20, 21, 22, 26, 27, or 28, wh rein said anti-target cell component (a) of said bifunctional heterodimeric antibody conjugate is one member selected from the group consisting of an anti-tumor cell antibody, an antibody to a virus infected c II, an antibody to a parasite infected cell, an antibody to an autoimmune cell, an antibody to activated cells that cause graft rejection and an antibody to activated cells that cause graft vs. host disease.

30. The method of Claim 19, 20, 21 or 22, wherein said antibody components (a) and (b) of the bifunctional heterodimeric antibody conjugate are joined by cross-liked complimentary reactive groups.

31. A method for selectively destroying target cell populations using a bifunctional antibody/cell surface-binding molecule conjugate which comprises (a) a molecular component other than an antibody capable of binding to target cell surface molecules and (b) an anti-lymphocyte antibody component wherein the anti-lyumphocyte antibody component is specific for activated lymphocytes, said method comprising:

(1) binding the bifunctional antibody/cell surface-binding molecule conjugate through the component (a) to target cells;

(2) activating lymphocytes; and

(3) binding the activated lymphocytes to the component (b) of the bifunctional antibody/cell surface-binding molecule conjugate;

wherein said steps (1), (2) and (3) can be conducted simultaneously or in any order, provided that step (2) is always conducted simultaneously with or prior to step (3).

32. The method of Claim 31, wherein the method comprises destroying the target cells in vitro.

33. The method of Claim 31, wherein the method comprises destroying the target cells in vivo.

34. The method of Claim 33, wherein the method comprises activating the lymphocytes in vivo or ex vivo.

35. The method of Claim 31, 32, 33, or 34, wherein the method comprises activating said lymphocytes with one member selected from the group consisting of antibodies, mitogens, antigens, and lymphokines.

36. The method of Claim 35, wherein the method comprises activating the lymphocytes with one or more antibodies.

37. The method of Claim 36, wherein the method comprises activating the lymphocytes with one or more antibodies selected from the group consisting of anti-T11$_2$, anti-T3, and anti-2H1.

38. The method of Claim 34, wherein the method comprises activating the lymphocytes with a mixtur of anti-T11$_2$ and anti-T11$_3$ antibodies.

39. The method of Claim 34, wherein said anti-lymphocyte antibody component (b) of said bifunctional antibody/cell surface-binding molecule conjugate is an anti-T11$_3$ antibody and said method comprises activating said lymphocytes in vivo or ex vivo with anti-T11$_2$.

40. The method of Claim 31, wherein said anti-lymphocyte antibody component (b) of said bifunctional antibody/cell surface-binding molecule conjugate is an anti-T11$_2$ antibody.

41. The method of Claim 31, 32, 33, 34, 38, 39, or 40, wherein said molecular component (a) of said bifunctional antibody/cell surface-binding molecule conjugate is capable of binding to one member selected from the group consisting of tumor cell surface molecules, virus infected cell surface molecules, parasite infected cell surface molecules, autoimmune cell surface molecules, surface molecules of activated cells that cause graft rejection and surface molecules of activated cells that cause graft vs. host disease.

42. The method of Claim 31, wherein said molecular component (a) of said bifunctional antibody/cell surface-binding molecule conjugate is one member selected from the group consisting of hormon s, lymphokines, and colony stimulating factors.

43. The method of Claim 31, 32, 33 or 34, wherein said components (a) and (b) of said bifunctional antibody/cell surface-binding molecule conjugate are joined by cross-linked complimentary reactive groups.

44. A method for selectively destroying target cell populations using a bifunctional hybrid antibody which comprises (a) an anti-target cell binding site component and (b) an anti-lymphocyte binding site component, wherein the anti-lymphocyte binding site component is specific for activated lymphocytes, said method comprising:

(1) binding the bifunctional hybrid antibody through the component (a) to target cells;

(2) activating lymphocytes; and

(3) binding the activated lymphocytes to the component (b) of the bifunctional hybrid antibody;

wherein said steps (1), (2) and (3) can be conducted simultaneously or in any order, provided that step (2) is always conducted simultaneously with or prior to step (3).

45. The method of Claim 44, wherein the method comprises destroying the target cells in vitro.

46. Th method of Claim 44, wherein the method comprises destroying th target cells in vivo.

47. Th method of Claim 46, wh rein the method comprises activating the lymphocytes $\underline{in}$ $\underline{vivo}$ or $\underline{ex}$ $\underline{vivo}$.

48. The method of Claim 44, 45, 46, or 47, wherein the method comprises activating said lymphocytes with one m mber selected from the group consisting of antibodies, mitog ns, antig ns, and lymphokines.

49. The method of Claim 48, wherein the method comprises activating the lymphocytes with one or more antibodies.

50. The method of Claim 49, wherein the method comprises activating the lymphocytes with one or more antibodies selected from the group consisting of anti-T11$_2$, anti-T3, and anti-2H1.

51. The method of Claim 47, wherein the method comprises activating the lymphocytes with a mixture of (i) a binding site specific for T11$_3$ and (ii) anti-T11$_2$ antibody.

52. The method of Claim 47, wherein the component (b) of said bifunctional hybrid antibody is a binding site specific for T11$_3$ and said method comprises activating said lymphocytes $\underline{in}$ $\underline{vivo}$ or $\underline{ex}$ $\underline{vivo}$ with anti-T11$_2$ antibody.

53. The method of Claim 44, wherein the component (b) of said bifunctional hybrid antibody is a binding site specific for T11$_2$.

54. The method of Claim 44, 45, 46, 47, 51, 52 or 53 wherein said anti-target cell binding site component (a) of said bifunctional hybrid antibody is specific for one member selected from the group consisting of tumor cells, virus infected cells, parasite infected cells, autoimmune cells, activated cells that cause graft rejection and activated cells that cause graft vs. host disease.

Relative Fluorescence Intensity